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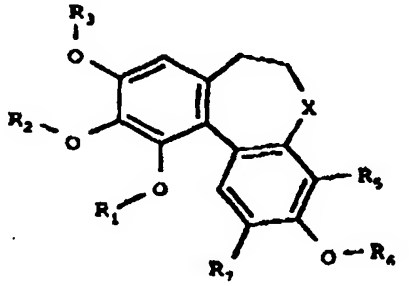
(54) Title: USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

(57) Abstract

Colchinol derivatives of formula (I) wherein R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2; X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4; R_4 is OH, O–alkyl or NR_8R_9; R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino; R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl or arylaminosulfonyl; and R_9 is H, alkyl or cycloalkyl and the pharmaceutically acceptable salts, solvates, and hydrates thereof have been found to be useful for treatment of diseases involving angiogenesis. Some of these compounds are novel. Particularly preferred are those compounds in which R_6 is PO_3H_2.



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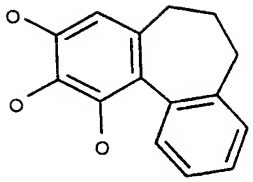
USE OF COLCHINOL DERIVATIVES AS VASCULAR DAMAGING AGENTS

This invention relates to vascular damaging agents and particularly to use in the preparation of agents for treatment of neovascularisation of a group of colchinol derivatives some of which are new compounds.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757 (1995)). For example, for a solid tumour to grow it must develop its own blood supply upon which it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Colchinol derivatives for example N-acetyl-colchinol are known. Anti-tumour effects have been noted on animal models (see for example - JNCI (Journal National Cancer Institute) Page 379-392 1952, Vol 13). However, the effect studied was that of gross damage (haemorrhage, softening and necrosis) and there is no suggestion of treatment of inappropriate angiogenesis by destruction of neovasculature.

A search of Chemical Abstracts (post 1955) based on the substructure

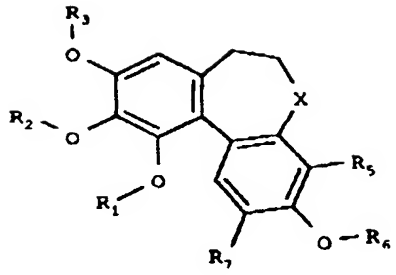


revealed a number of colchinol related structures.

To the extent that any of these compounds have been studied for anti-cancer activity it is because tubulin binding agents might be expected to be anti-mitotic and therefore to have a direct effect on tumour cells.

In the course of the work on the present invention, the issue of the relevance of tubulin-binding properties to possible effectiveness as anti-vascular agent was studied but no predictability was found. Thus docetaxel (Lancet, 344, 1267-1271, 1994), which is a tubulin-binding agent, had no vascular-damaging effects even when administered at its Maximum Tolerated Dose. Even when the present inventors tested some compounds structurally related to the present invention, the therapeutic window (ratio of MTD (Maximum tolerated dose) to MED (Minimum effective dose)) was found to be too small for potential clinical effectiveness.

According to the present invention there is provided the use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;

X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4

R_4 is OH, O-alkyl or NR_8R_9;

R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;

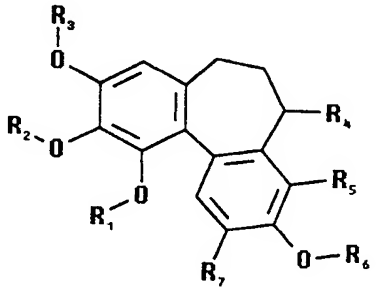
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl

and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

It is believed, though this is not limiting on the invention, that the use of compounds of the invention damages newly-formed vasculature, for example the vasculature of tumours, thus effectively reversing the process of angiogenesis as compared to known anti-angiogenic agents which tend to be less effective once the vasculature has formed.

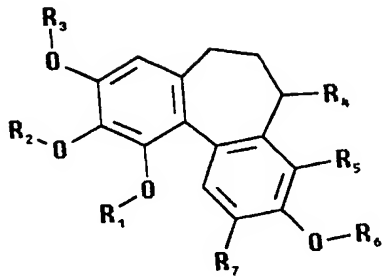
Certain of these compounds are novel. In one embodiment the novel compounds are those of formula I in which at least one of R_1, R_2, R_3, R_6 is PO_3H_2. In a particular preferred embodiment R_6 is PO_3H_2. Particularly preferred are compounds defined by the formula



II

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;
R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

In another aspect of the invention the novel compounds are of formula



IIA

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;
R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl or hydroxyethyl, or acetoxyethyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

Preferred compounds used in the invention and of the invention are those in which R_1, R_2 and R_3 are alkyl and

those in which R_4 is acylamino.

As used herein the term "alkyl", (including any aliphatic structure chain related to alkyl) means a straight or branched-chain group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. Optional substituents which may be present on the alkyl groups include one or more substituents selected from halogen, amino, monoalkylamino, dialkylamino, hydroxy, alkoxy, alkylthio, alkylsulphonyl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, carboxyl, sulphate or phosphate groups. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

An alkenyl group is an olefinic group containing from two to seven carbon atoms for example methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene, s-butylene and t-butylene. An alkynyl group is of a group of 2-7 carbon atoms for example ethynyl, propynyl or butynyl group.

The term aryl alone or in combination means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents examples of which are halogen, alkyl, haloalkyl, hydroxy, nitro, cyano, amino and alkoxy. A haloalkyl group can carry one or more halogen atoms with the examples of such groups being trifluoromethyl and dichloromethyl.

The term heteroaryl is defined herein as a mono- or bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms and a maximum of 9 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl,

pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups.

The term aralkyl is defined herein as an alkyl group, as previously defined, in which one of the hydrogen atoms is replaced by an aryl or heteroaryl group as defined herein.

Where one or more functional groups in compounds of formulae I, II, IIA are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hrdrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formulae I, II, IIA may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers and mixtures thereof.

One useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl.

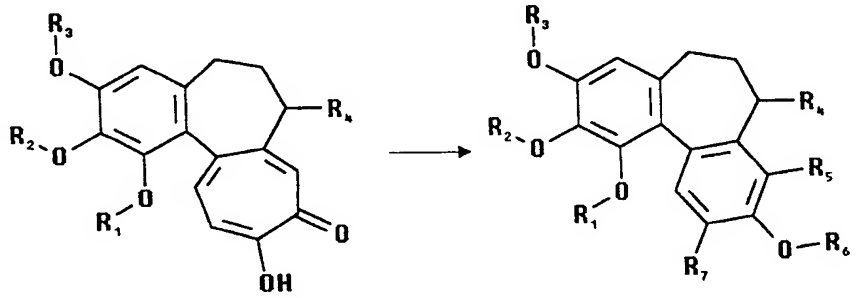
Another useful group of compounds includes those in which R_1, R_2 and R_3 are each alkyl and R_5 and R_7 are each hydrogen. A particularly useful subset of this group includes compounds in which R_1, R_2 and R_3 are each methyl and R_6 is hydrogen, alkyl or PO_3H_2.

Particularly useful compounds according to the invention include:

N-Acetylcolchinol-O-phosphate and its salts, solvates and hydrates.

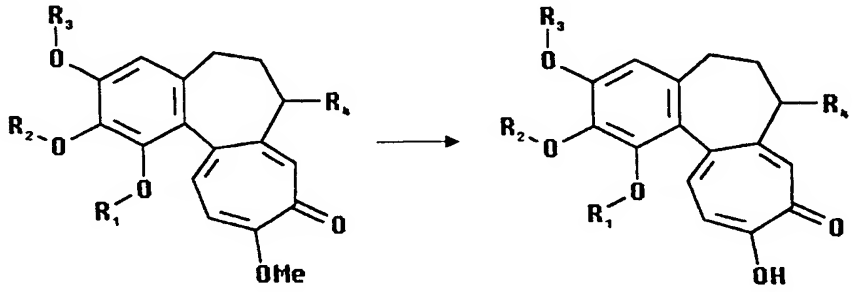
Compounds of formulae I, II or IIA may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description the symbols R_1, R_2, R_3, R_4, R_5, R_6 and R_7 when used in the formulae depicted are to be understood to represent those groups described above in relation to formulae I, II or IIA unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Thus according to a further aspect of the invention compounds of formulae II or IIA in which R_5, R_6 and R_7 are each hydrogen may be prepared by treatment of a compound of formula (2) with alkaline hydrogen peroxide. The reaction may be conveniently performed in aqueous sodium hydroxide solution in the absence or presence of a cosolvent such as an alcohol, for example ethanol, at a temperature in the range for example 0- 100°C preferably at or near to 60°C.



(2) II or IIA

Intermediates of formulae (2) may be prepared by acid hydrolysis of compounds of formulae (3); The reaction is conveniently carried out in an aqueous acid such as hydrochloric acid at an elevated temperature, for example at or near 100°C.



(3) (2)

Compounds of formula (3) are either known or can be prepared from colchicine by conventional procedures.

Compounds of formulae I, II or IIA may also be prepared from other compounds of formulae I, II or IIA, by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, arylation, heteroarylation, acylation, thioacylation, sulphonylation, sulphation, phosphorylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formulae I, II or IIA may be modified by, for example oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formulae I, II or IIA.

Thus for example a compound of formulae II or IIA containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a

tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formulae II or IIA may be sulphonylated by treatment with, for example, an alkyl or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C, conveniently at or near ambient temperature.

In a further general example a compound of formulae II or IIA containing a hydroxy group can be converted into the corresponding dihydrogenphosphate ester by treatment with for example di-tert-butyl diethylphosphoramidite in the presence of a suitable catalyst for example tetrazole. In a solvent such as an ether solvent for example tetrahydrofuran at a temperature in the range -40 to 40°C, conveniently at or near room temperature, followed by treatment with an oxidising agent for example 3-chloroperoxy benzoic acid at a temperature in the range -78°C to 40°C preferably -40 to -10°C. The resulting intermediate phosphate triester is treated with an acid for example trifluoroacetic acid in a solvent such as a chlorinated solvent e.g. dichloromethane at a temperature in the range -30 to 40°C conveniently at or near 0°C to give the compound of formula (2) containing a dihydrogenphosphate ester.

In a further general example a compound of formula (2) containing an amide can be hydrolysed by treatment with

for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C.

In a further general example compounds of formulae II or IIA may be alkylated by reaction with a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of around -10 to 80°C.

Preparation of a compound of formulae II or IIA as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

Acid addition salts of the compounds of formulae II or IIA are prepared in a conventional manner by treating a solution or suspension of the free base II or IIA with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formulae I, II or IIA derived from inorganic or organic bases are prepared in a conventional manner by treating a solution or suspension

of the free acid I, II or IIA with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

Compounds according to the invention are able to destroy tumour vasculature and vasculature that has been newly formed while leaving unaffected normal, mature vasculature. The ability of the compounds to act in this way may be determined by the tests described in the Examples hereinafter.

The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide, antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example asparaginase; topoisomerase inhibitors for example etoposide, topotecan

and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following tests were used to demonstrate the activity and selectivity of compounds according to the invention.

Activity against tumour vasculature measured by radioactive tracer.

The following experiment demonstrates the ability of the compounds to damage selectively tumour vasculature.

Subcutaneous CaNT tumours were initiated by injecting 0.05ml of a crude tumour cell suspension, approximately 10^6 cells, under the skin overlying the rear dorsum of 12-16 week-old mice. The animals were selected for treatment after approximately 3-4 weeks, when their tumours reached a geometric mean diameter of 5.5-6.5mm. Compounds were dissolved in sterile saline and injected intraperitoneally in a volume of 0.1 ml per 10 g body weight. Tumour perfusion was measured 6 hours after intraperitoneal administration in tumour, kidney, liver, skin muscle, gut and brain by the ^{86}RbCI extraction technique (Sapirstein, Amer J Physiol, 193, 161-168, 1958). Tissue radioactivity measured 1 minute after an intravenous injection of ^{86}RbCI was used to calculate relative blood flow as a proportion of cardiac output (Hill and Denekamp, Brit J Radiol, 55, 905-913, 1982). Five animals were used in control and treated groups. Results were expressed as a percentage of the blood flow in the corresponding tissues in vehicle treated animals.

Activity against tumour vasculature measured by fluorescent dye.

The following experiment further demonstrates the ability of the compounds to damage tumour vasculature.

Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the florescent dye Hoechst 33342

according to the method of Smith *et al* (Brit J Cancer 57, 247-253, 1988). Five animals were used in control and treated groups. The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 6 hours after intraperitoneal drug treatment. One minute later, animals were killed and tumours excised and frozen; 10μm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, 4, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels. Compounds of the invention reduced tumour functional vascular volume by greater than 20% at doses of 50mg/kg or below.

The following non-limiting Examples illustrate the invention. In the Examples all ^{1}Hnmr were run at 300MHz unless otherwise specified. Column chromatography was performed on silica gel. All temperatures are in °C. The following abbreviations are used: THF - tetrahydrofuran; DMSO - dimethylsulphoxide; MCPBA - 3-chloroperoxybenzoic acid.

EXAMPLE 1

N-Acetylcolchinol-O-phosphate

A solution of N-acetylcolchinol (260mg, 0.76 mmol) in anhydrous THF (2ml) under an atmosphere of nitrogen was treated with di-t-butyl diethylphosphoramidite (189mg, 0.75mmol) and 1(H)-tetrazole (0.14g, 1.99mmol) and the solution stirred at 20° for 0.5h. The solution was cooled to -40° and a solution of 85% MCPBA (202mg, 0.99mmol) in anhydrous dichloromethane (2ml) at such a rate that the temperature remained below -10°. The solution was allowed to warm to room temperature, diethyl

ether (30ml) was added and the resulting solution washed successively with 10% aqueous sodium metabisulphite (two 25ml portions), 5% aqueous sodium bicarbonate (two 25ml portions), 5% aqueous citric acid (30ml), 5% aqueous sodium bicarbonate, and brine. The organic solution was concentrated under reduced pressure and the residue subjected to column chromatography to afford a white foam (170mg) containing N-acetylcolchinol-O-di-*t*-butylphosphate which was redissolved in dichloromethane (5ml), cooled to 0° and treated with trifluoroacetic acid (0.5ml).

The solution was allowed to warm to room temperature and stir 1 hr before being concentrated under reduced pressure and triturated with ether to give the title compound (110mg) as a white solid m.p. 233-235°. δH (d6-DMSO) 8.38(d,1H, J=8Hz), 7.27(d, 1H,J=7Hz), 7.12(d, 1H, J=8Hz), 7.10(s,1H), 6.77 (s, 1H), 4.48 (m, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.49 (s, 3H), 2.5 (signal partially obscured by DMSO peak), 1.9-2.2 (m, 2H), 1.86 (s, 3H).

The activity of the phosphate compound was measured by the radioactive tracer assay described above: the compound of this Example gave a 65% decrease in tumour blood flow at a dose of 125mg/kg with no significant reduction in blood flow in skin, muscle, liver, kidney, gut or heart.

The phosphate compound was compared with the parent N-acetylcolchinol for the maximum tolerated dose (MTD) (no deaths in three animals), minimum effective dose (MED) measured by the fluorescent dye technique already described and the therapeutic window (MTD/MED).

	MTD mg/Kg body weight	MED mg/Kg body weight	Therapeutic Window (MTD/MED)
N-acetyl colchinol	125	30	4
N-acetyl colchinol-O-phosphate	750	50	15

Though the phosphate had a slightly higher MED the "window" was significantly greater. This was unexpected. The phosphate also gave greater solubility.

For comparison the "therapeutic windows" for colchicine (the closest structure to the present compounds and docetaxel (a tubulin-binding drug, marketed as "Taxotere", which has no vascular-damaging activity) and these data are presented in the following table:

Table 1 - Therapeutic windows of other tubulin-binding agents (by fluorescent dye technique)

Compound	MED (mg/kg body weight)	MTD (mg/kg body weight)	MTD/MED
Docetaxel	>30(No effect at 30)	30	<1
Colchicine	2.5	5	2

EXAMPLE 2

N-Ethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in THF (15ml) was added dropwise over 15 minutes to a suspension of lithium aluminum hydride (106mg, 2.74mmol) in THF (10ml) with ice-bath cooling. The mixture was heated at reflux for 15h, allowed to cool and treated with further lithium aluminium hydride (53mg, 1.4mmol) before heating at reflux for a further 3h. The mixture was cooled (ice bath) and water (10ml) added dropwise before extraction with three portions of ethyl acetate. The combined,

dried (MgSO4) extracts were concentrated under reduced pressure to a green gum which, on trituration with ether, gave the title compound as a light-green solid. m.p. 185°C (dec.), m/e 343 (M+). Anal. Calculated for $C_{20}H_{25}NO_4$ H_2O: C, 66.46; H, 7.53; N, 3.88. Found: C,66.50; H, 7.17; N, 3.79.

EXAMPLE 3

N-Benzyloxycarbonylcolchinol

A solution of colchinol (625mg, 1.98mmol) in dry pyridine (10ml) was treated dropwise with benzylchloroformate (0.566ml, 3.97 mmol) and the mixture stirred 16h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 50% ethyl acetate/petroleum ether. The resultant orange gum was crystallised from ether/petroleum ether to give the title compound (346mg) as a pale yellow solid. m.p. 79-81°C, m/e 449 (M+). Anal. Calculated for $C_{26}H_{27}NO_6$ $0.33H_20$; C, 68.57; H, 6.07; N, 3.08. Found: C,68.71; H, 6.18; N, 2.91.

EXAMPLE 4

N-(Phenylcarbamoyl) colchinol

A solution of colchinol (400mg, 1.27mmol) in dry pyridine (10ml) was treated dropwise with phenyl isocyanate (0.151ml, 1.39mmol) and the mixture stirred for 18h before heating at reflux for 2h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with 35% ethyl acetate/petroleum ether. The resultant gum was crystallised from ether/petroleum ether to give the title

compound (261mg) as a pale orange solid. m.p. 145-146°C, m/e 434 (M+).

EXAMPLE 5

N-Mesylcolchinol

A solution of N,O-dimesylcolchinol (234mg, 0.5mmol) in methanol (8ml) was treated with sodium hydroxide (40mg, 1mmol) and the mixture heated at reflux for 3h. Solvent was removed under reduced pressure and water (5ml) added. The solution was rendered neutral by the addition of 1M hydrochloric acid and extracted with three portions of dichloromethane. The combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give the title compound (123mg) as a pink solid. m.p. 234-236°C, m/e 393 (M+).

The N,O-dimesylcolchinol used as starting material was prepared as follows: A solution of colchinol (500mg, 1.6mmol) in dry pyridine (15ml) was treated with mesyl chloride (0.135ml, 1.7mmol) and the mixture stirred at room temperature 36h. A further portion of mesyl chloride (0.135ml, 1.7mmol) was added and stirring continued 16h. Solvent was removed under reduced pressure and water (5ml) added. The solution was extracted with three portions of chloroform and the combined, dried ($MgSO_4$) extracts were concentrated under reduced pressure to give a brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give N,O-dimesylcolchinol (292mg) as a light orange solid.

EXAMPLE 6

N-Dimethylsulphamoylcolchinol

A solution of colchinol (50mg, 0.16mmol) in dry acetonitrile (3ml) and triethylamine (0.022ml, 0.16mmol) was treated with dimethylsulphamoyl chloride and the mixture stirred for 30 minutes before heating at reflux

for 15h. Solvent was removed under reduced pressure, water added and the resulting mixture extracted with three portions of chloroform. The combined, dried (Na_2SO_4) extracts were concentrated under reduced pressure to a dark brown gum which was subjected to column chromatography on silica gel eluting with ethyl acetate to give the title compound (46mg) as a pale orange gum which solidified. m.p. 82-85°C m/e 422 (M+).

EXAMPLE 7

N-Acetyl-O-methoxycarbonylmethylcolchinol

A solution of N-acetylcolchinol (500mg, 1.4mmol) in dry DMF (5ml) at 0°C was treated with methylbromoacetate (322mg, 2.1mmol) and sodium hydride (84mg of a 60% suspension in oil, 2.1mmol) and the mixture stirred for 30 minutes. Water (50ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed successively with four portions of water and two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and solvent removed under reduced pressure to give the title compound (280mg) as a white solid m.p. 82-83°C. m/e 429 2 (M+). Anal. Calculated for $C_{23}H_{27}NO_7$ $0.33H_2O$; C, 63.45; H, 6.40; N, 3.22. Found: C, 63.53; H, 6.29; N, 3.17.

EXAMPLE 8

N-Acetyl-O-carboxymethylcolchinol

A solution of N-acetyl-O-methoxycarbonylmethylcolchinol (140mg, 0.33mmol) in acetonitrile (5ml) was treated with aqueous potassium hydroxide solution (1.0M, 5ml) and the mixture heated at 80°C for 30 minutes. The cooled mixture was adjusted to pH3 by addition of 2M hydrochloric acid and extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced

pressure. Addition of acetone (2ml) and hexane (1ml) produced the title compound (58mg) as a white solid m.p. 220-221°C. m/e 415.3 (M+). Anal. Calculated for $C_{22}H_{25}NO_7$ $0.33H_2O$; C,62.71; H, 6.14; N, 3.32. Found: C, 62.63; H, 6.02; N, 3.26.

EXAMPLE 9

N-Acetyl-O-cyclopentylcolchinol

A solution of N-acetylcolchinol (200mg, 0.56mmol) in dry DMF (2ml) at 0°C was treated with sodium hydride (33mg of a 60% suspension in oil, 0.84mmol) followed by cyclopentyl bromide (125mg, 0.84mmol) and the mixture stirred 1h. A further portion of sodium hydride (17mg of a 60% suspension in oil, 0.42mmol) and of cyclopentyl bromide (63mg, 0.42mmol) and the mixture stirred overnight at room temperature. Water (10ml) was added and the mixture extracted with four portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium chloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. The title compound (160mg) was obtained as a white solid m.p. 89-94°C. m/e 425.3 (M+). Anal. Calculated for $C_{25}H_{31}NO_5$: C,70.54; H, 7.35; N, 3.29. Found: C, 70.55; H, 7.35; N, 3.25.

EXAMPLE 10

N-Acetyl-10-nitrocolchinol

A solution of N-acetylcolchinol (100mg, 0.27mmol) in glacial acetic acid (20ml) was treated slowly with 20ml of a solution of concentrated nitric acid (0.34ml) in acetic acid (100ml) keeping the temperature at about 12°C. The mixture was stirred at room temperature for 18h, a further 1ml of the nitric acid/acetic acid solution added and stirring continued for 2h. The mixture was poured onto ice and extracted with three portions of ethyl acetate. The combined extracts were washed with two portions of saturated aqueous sodium

cloride solution, dried ($MgSO_4$) and concentrated under reduced pressure. Purification on silica gel eluting with ethyl acetate gave the title compound (50mg) as a pale yellow solid m.p. 117-8°C. m/e 401.9 (M+) Anal. Calculated for $C_{20}H_{22}N_2O_7$ $0.33H_2O$; C, 58.82; H, 5.56; N, 6.86. Found: C, 58.87; H, 5.66; N, 6.55.

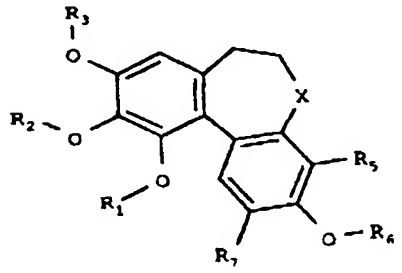
EXAMPLE 11

The activity against tumour vasculature was measured by the fluorescent dye technique described above for the compounds of Examples 1-10 administered at 50mg/kg and N-acetylcolchinol

Compound of Example	% decrease in vascular volume
1	89
2	38
3	43
4	37
5	38
6	30
7	12
8	49
9	59
10	28
N-acetylcolchinol	78

CLAIMS:

1. The use of colchinol derivatives for the preparation of compositions for the treatment of diseases involving angiogenesis in which the colchinol derivative has the formula



I

wherein

R_1, R_2, R_3 and R_6 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, alkanoyl, PO_3H_2;
X is carbonyl (CO), thiocarbonyl (CS), methylene (CH_2) or a group CHR_4
R_4 is OH, O-alkyl or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, hydroxy, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl
and the pharmaceutically acceptable salts, solvates, and hydrates thereof.

2. A use according to claim 1 wherein at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

3. A use according to claim 2 wherein R_6 is PO_3H_2.

4. A use according to any one of claims 1 to 3 in which R_1, R_2 and R_3 are alkyl.

5. A use according to any one of claims 1 to 4 in which R_4 is acylamino.

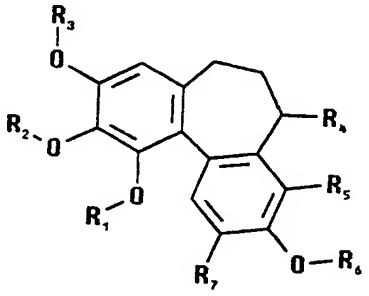
6. A use according to claim 1 in which R_6 is PO_3H, R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

7. Compounds of formula I in which at least one of R_1, R_2, R_3 and R_6 is PO_3H_2.

8. A compound according to claim 7 in which R_6 is PO_3H_2.

9. A compound according to claim 8 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

10. A compound according to claim 7 of formula



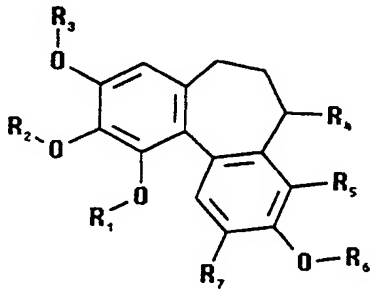
II

wherein

R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl, or PO_3H_2;

R_6 is PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, alkoxy, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;
and R_9 is H, alkyl or cycloalkyl,
and the pharmaceutically acceptable salts, solvates and hydrates thereof.

11. A compound of the formula



IIA

wherein
R_1, R_2 and R_3 are each independently H, optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, alkanoyl or PO_3H_2;
R_6 is H, optionally substituted alkyl, cycloalkyl, alkenyl, akynyl or PO_3H_2;
R_4 is H or NR_8R_9;
R_5 and R_7 are each independently H, alkyl, halogen, nitro or amino;
R_8 is H, optionally substituted alkyl, cycloalkyl, alkanoyl, thioalkanoyl, aryl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, aryloxycarbonyl,

aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylaminocarbonyl, alkylsulphonyl, arylsulphonyl, aminosulphonyl, alkylamnosulphonyl, dialkylaminosulphonyl or arylaminosulphonyl;

and R_9 is H, alkyl or cycloalkyl, with the proviso that, when R_1, R_2 and R_3 are all methyl groups and R_4 is hydrogen, acetylamino, acetylmethylamino, amino, methylamino or dimethylamino then R_6 is not hydrogen, methyl, hydroxyethyl, or acetoxyethyl,

and the pharmaceutically acceptable salts, solvates and hydrates thereof.

12. A compound according to claim 11 in which R_1, R_2 and R_3 are alkyl and R_4 is acylamino.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/GB 98/01977

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/66 A61K31/165 A61K31/27 C07C13/547

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	O. BOYE ET AL.: "Synthesis of carbon-14 labeled electrophilic ligands of the colchicine binding site of tubulin: chloroacetates of demethylthiocolchicines and of N-acetylcolchinol, isothiocyanates of 9-deoxy-N-acetylcolchinol." J. LABELLED COMPD. RADIOPHARM., vol. 33, no. 4, 1993, pages 293-299, XP002081866 ---	
A	R. BRECHT ET AL.: "Dihydrocolchicine 8,12-endoperoxide. a novel starting material for convenient syntheses of the allocolchicinoids N-acetylcolchinol O-methyl ether and androbiphenyline." LIEBIGS ANN., no. 11, 1997, pages 2275-2279, XP002081867 --- -/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 1998	11/11/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GIL-JONG KANG ET AL: "n-acetylcolchinol O-methyl ether and thiocolchicine, potent nalogs of colchicine modified in the C-ring" J. BIOL. CHEM., vol. 265, no. 18, 1990, pages 10255-10259, XP002081868 ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13831f XP002081869 & JP 39 019635 B (SANKYO CO.) see abstract ---	
A	DATABASE WPI Week 6800 Derwent Publications Ltd., London, GB; AN 66-13830f XP002081870 & JP 39 019634 B (SANKYO CO.) see abstract -----	

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